

# Competent cell

## *Materials and Equipment*

- Alcohol lamp
- Microtube rack
- Lighter
- Micropipette
- Micropipette tips
- Microtubes (0.6 ml)
- Centrifuge
- Sterile pipettes
- Laminar flow hood

## *Reagents*

- LB medium
- CaCl<sub>2</sub> 0.1M
- CaCl<sub>2</sub> 0.1M-15% glycerol
- Bacterial strain

## *Methodology*

1. Seed culture ON (37°C) of the bacterial strain. Inoculate a flask with seed culture until OD reaches 0.5-0.6 at 600 nm. Centrifuge medium, for the obtention of the bacterial pellet.
2. Remove the supernatant (culture media), and resuspend in 10 ml of CaCl<sub>2</sub> 0.1M (cold), incubate in ice for 10 minutes.
3. Centrifuge at 4000 rpm at 4°C for 10 minutes.
4. Discard the supernatant and resuspend in 2 ml of CaCl<sub>2</sub> 0.1M-15% glycerol. Incubate for 10 minutes in ice.
5. Make aliquots of 200µl and store at -70°C